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Dietary Silicon and Arginine Affect Mineral Element Composition of Rat Femur and Vertebra

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ABSTRACT

Both arginine and silicon affect collagen formation and bone mineralization. Thus, an experiment was designed to determine if dietary arginine would alter the effect of dietary silicon on bone mineralization and vice versa. Male weanling Sprague–Dawley rats were assigned to groups of 12 in a 2 × 2 factorially arranged experiment. Supplemented to a ground corn/casein basal diet containing 2.3 µg Si/g and adequate arginine were silicon as sodium metasilicate at 0 or 35 µg/g diet and arginine at 0 or 5 mg/g diet. The rats were fed ad libitum deionized water and their respective diets for 8 wk. Body weight, liver weight/body weight ratio, and plasma silicon were decreased, and plasma alkaline phosphatase activity was increased by silicon deprivation. Silicon deprivation also decreased femoral calcium, copper, potassium, and zinc concentrations, but increased the femoral manganese concentration. Arginine supplementation decreased femoral molybdenum concentration but increased the femoral manganese concentration. Vertebral concentrations of phosphorus, sodium, potassium, copper, manganese, and zinc were decreased by silicon deprivation. Arginine supplementation increased vertebral concentrations of sodium, potassium, manganese, zinc, and iron. The arginine effects were more marked in the silicon-deprived animals, especially in the vertebra. Germanium

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concentrations of the femur and vertebra were affected by an interaction between silicon and arginine; the concentrations were decreased by silicon deprivation in those animals not fed supplemental arginine. The change in germanium is consistent with a previous finding by us suggesting that this element may be physiologically important, especially as related to bone DNA concentrations. The femoral and vertebral mineral findings support the contention that silicon has a physiological role in bone formation and that arginine intake can affect that role.

Index Entries: Silicon; bone mineralization; trace elements; bone DNA.

INTRODUCTION

Arginine is an essential amino acid for the rat. In animals, L-arginine apparently induces growth hormone and insulin-like growth factor-1 responses and stimulates nitric oxide synthase. Growth hormone and insulin-like growth factor-1 are important mediators of bone turnover and osteoblastic bone formation, whereas nitric oxide is a potent inhibitor of osteoclastic bone resorption *(1).* By affecting these physiological regulators of bone remodeling, L-arginine could potentially increase bone formation over bone resorption and, consequently, increase bone mass.

There is experimental evidence suggesting that arginine supplementation promotes bone formation. A mixture of lactose, L-arginine, and L-lysine improved fracture healing of rabbits subjected to an osteotomy of the left fibula *(2).* These authors suggested that arginine was involved not only in the increase of intestinal calcium absorption but also in collagen synthesis. Although there is evidence that L-arginine affects bone maintenance, minimal attention has been given to the possible interaction between arginine and other macro and/or trace minerals, including silicon, associated with mineralized bone formation and remodeling.

Silicon can affect bone formation and remodeling *(3).* The basic amino acids such as arginine can increase silicon absorption *(4).* Therefore, the effects of silicon on bone mineralization may be modified by the amount of arginine in the diet. Thus, we performed an experiment to ascertain whether supplemental arginine compared to adequate arginine would affect bone mineral composition differently in silicon-deprived and silicon-adequate rats (i.e., whether the intake of additional arginine would affect the response of rats to silicon deprivation).

MATERIALS AND METHODS

Forty-eight weanling male Sprague–Dawley rats, aged 21 d (Sasco, Omaha, NE), were weighed upon arrival and housed three per all-plastic cage measuring 50 × 24 × 16 cm *(5)* and located inside a laminar airflow

rack (Lab Products, Maywood, NJ). Rats were randomly assigned to treatment groups with no significant differences in weight (mean of 39 g) in a 2 × 2 factorial arrangement. The independent variables, or factors, were supplements per gram of fresh diet of silicon (as sodium metasilicate) at 0 (–Si) or 25 µg (+Si) and arginine at 0 or 5 mg. Sodium metasilicate was reagent grade (J. T. Baker, Phillipsburg, NJ) and arginine was obtained from Ajinomoto U.S.A. (Teaneck, NJ).

The composition of the basal diet has been reported *(6)*. Analysis indicated that the basal diet contained 2.3 µg Si/g. Fresh food in plastic cups was provided ad libitum each day. The diets were mixed 3 d before the start of the experiment. The diets were not pelleted and were stored at –16°C in tightly capped plastic containers. The rats were provided deionized water (Super Q System, Millipore Corp., Bedford, MA) in plastic cups. Absorbent paper under the false-bottom cages was changed daily. Room temperature was maintained at 23°C. Room lighting was controlled automatically to provide 12 h each of light and dark. Animals were weighed and provided clean cages weekly.

The rats were fed their respective diets for 8 wk. Following a 16-h fast, animals were weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination with a heparin-coated syringe and needle. The right rear leg and a vertebra section were removed from each rat and frozen at –20°C for 3–4 wk until the femur and vertebra were analyzed for mineral content and the tibia was analyzed for DNA content.

To determine the DNA concentration of bone, tibias were demarrowed and pulverized by freezing in liquid nitrogen and striking with a hard object while in metal containers. The pulverized bone was lyophilized before extracting the DNA by shaking in 1 : 9 dilution (w/v) of 0.1 *N* NaOH for 24 h. The DNA of an aliquot spotted in a Falcon tube was determined flourometrically by using 3,5-diaminobenzoic acid *(7)*. Alkaline phosphatase, albumin, and creatinine concentrations in plasma were measured by colorimetric methods (Sigma Chemical Co., St. Louis, MO). Plasma creatine was chemically changed to form all creatinine for the creatinine measurement.

The air-dried basal diets and vacuum-dried bone samples were ashed in platinum crucibles at 450°C. A lithium–boron fusion technique was used to ash the diets *(8)*. Plasma, femur, vertebra, and dietary macrominerals and trace elements were determined by inductively coupled argon plasma atomic emission *(9)*. Standard reference materials (National Institute of Standards and Technology, Gaithersburg, MD) #1572 Citrus Leaves and #1577A Bovine Liver were used as quality control materials in the analyses of minerals. Replicate analysis of Citrus Leaves yielded (µg/g) values of 31529 ± 157 calcium, 17.5 ± 0.3 copper, 88.1 ± 0.3 iron, 15645 ± 74 potassium, 5259 ± 32 magnesium, 23.7 ± 0.3 manganese, 143.9 ± 0.8 sodium, 1308 ± 24 phosphorus, and 29.2 ± 0.6 zinc compared to certified values of 31500 ± 1000, 16.5 ± 1.0, 90 ± 10, 18200 ± 600, 5800 ± 300, 23 ± 2, 160 ± 20, 1300 ± 200, and 29 ± 2, respectively.

Data were statistically compared by using two-way analysis of variance *(10).* Tukey's Studentized range tests were performed when appropriate. Differences between values were considered significant at $p \leq 0.05$.

RESULTS

As shown in Table 1, growth and the liver weight/body weight ratio of the rats were significantly decreased by silicon deprivation. Arginine neither affected growth nor the liver weight/body weight ratio. Although silicon did not alter the kidney weight/body weight ratio, this ratio was significantly increased by supplemental arginine.

The data in Table 1 also show that silicon deprivation was achieved because, compared to animals fed the silicon-adequate diet, those fed the silicon-inadequate diet had lower plasma concentrations of silicon (p=0.004) and a higher plasma alkaline phosphatase activity (p=0.03.) An interaction between silicon and arginine affected plasma albumin. In –Si animals, supplemental arginine decreased plasma albumin; in contrast, supplemental arginine increased the plasma albumin in +Si rats. Dietary silicon did not affect the plasma creatine concentration, but arginine supplementation significantly increased it (p=0.0001). Neither plasma total protein nor blood urea nitrogen were significantly affected by the dietary variables; thus, these data are not shown.

An interaction between silicon and arginine affected tibia DNA (Table 2). When dietary silicon was adequate, the concentration of DNA in the tibia was markedly lower in arginine-supplemented rats than in arginine-unsupplemented rats. In contrast, the tibia DNA concentration of the rats fed the silicon-inadequate diet was slightly increased by supplemental arginine.

Silicon deprivation significantly decreased the concentrations of calcium, potassium, and sodium in the femur (Table 2). The only macromineral affected by arginine was sodium, and this was through an interaction with silicon. Arginine supplementation increased the sodium concentration in –Si rats and decreased the concentration in +Si rats.

Table 2 shows that concentrations of the trace elements zinc, copper, and manganese were significantly decreased in the femur by silicon deprivation. Supplemental arginine did not affect femur zinc and copper concentrations, but increased the manganese and decreased the molybdenum concentrations in the femur. No treatment affected femur iron. An interaction between silicon and arginine affected femur germanium concentrations (Table 2). When dietary silicon was adequate, the femur concentration of germanium was lower in the arginine-supplemented rats than in the arginine-unsupplemented rats. In contrast, in animals fed a silicon-inadequate diet, arginine supplementation increased femur germanium concentrations.

The most evident impact of silicon on vertebra mineralization (Table 3) is shown by the concentrations of the macrominerals, phosphorus, potassium, and sodium. Concentrations of these three minerals decreased in

Table 1
Effect in Rats of Silicon, Arginine, and Their Interaction on Growth, Liver Weight/Body Weight Ratio, Kidney Weight/Body Weight Ratio, and Plasma Silicon, Alkaline Phosphatase, Albumin, and Creatinine

Treatment Si µg/g	Arg g/kg	Body Weight (g)	Liver Weight/ Body Weight	Kidney Weight/ Body Weight	Plasma Silicon µg/mL	Plasma Alkaline Phosphatase µmoles/min/mL x 10	Plasma Albumin g/dL	Plasma Creatinine mg/dL
0	0	297 ± 9[a]	2.75 ± 0.07	0.30 ± 0.01	0.28 ± 0.02	0.86 ± 0.05	0.85 ± 0.03	2.46 ± 0.36
0	5	294 ± 9	2.68 ±0.07	0.31 ± 0.01	0.25 ± 0.02	0.84 ± 0.05	0.77 ± 0.03	4.65 ± 0.36
35	0	322 ± 9	2.88 ± 0.07	0.30 ± 0.01	0.31 ± 0.02	0.77 ± 0.05	0.81 ± 0.03	3.32 ± 0.36
35	5	320 ± 9	2.81 ± 0.07	0.32 ± 0.01	0.32 ± 0.02	0.72 ± 0.05	0.88 ± 0.03	4.77 ± 0.36
Analyses of Variance – P values								
Si		0.008	0.05	NS	0.004	0.03	NS	NS
Arg		NS	NS	0.05	NS	NS	NS	0.0001
Si x Arg		NS	NS	NS	NS	NS	0.04	NS

[a] Least square (LS) means ± standard error LS means (n=12).

Table 2
Effect in Rats of Dietary Arginine and Silicon on Tibia DNA and Femur Mineral Concentrations

Treatment Si µg/g	Arg g/kg	DNA mg/g	Ca mg/g	P mg/g	K mg/g	Na mg/g	Zn µg/g	Cu µg/g	Mn µg/g	Fe µg/g	Mo µg/g	Ge µg/g
0	0	4.41 ± 0.18[a]	212 ± 6	109 ± 4	4.02 ± 0.18	4.08 ± 0.13	165 ± 6	1.09 ± 0.07	0.67 ± 0.02	71 ± 4	2.15 ± 0.14	0.67 ± 0.98
0	5	4.64 ± 0.16	225 ± 7	116 ± 4	4.07 ± 0.18	4.33 ± 0.13	184 ± 5	1.18 ± 0.07	0.73 ± 0.02	75 ± 4	1.70 ± 0.14	1.49 ± 1.03
35	0	4.87 ± 0.17	245 ± 6	117 ± 4	4.43 ± 0.17	5.06 ± 0.13	200 ± 5	1.33 ± 0.08	0.75 ± 0.02	73 ± 4	2.23 ± 0.14	4.91 ± 0.98
35	5	4.18 ± 0.18	252 ± 6	117 ± 4	4.58 ± 0.18	4.79 ± 0.13	199 ± 5	1.43 ± 0.07	0.78 ± 0.02	71 ± 4	2.14 ± 0.14	0.64 ± 0.64
Si		NS	0.0001	NS	0.02	0.0001	0.0001	0.002	0.001	NS	NS	NS
Arg		NS	NS	NS	NS	NS	NS	NS	0.03	NS	0.05	NS
Si x Arg		0.01	NS	NS	NS	0.05	NS	NS	NS	NS	NS	0.02

[a] Least square (LS) means ± standard error LS means.

Table 3
Effect in Rats of Silicon, Arginine, and Their Interaction on Vertebra Mineral Concentrations

Treatment Si μg/g	Arg g/kg	Ca mg/g	P mg/g	K mg/g	Na mg/g	Zn μg/g	Cu μg/g	Mn μg/g	Fe μg/g	Mo μg/g	Si μg/g	Ge μg/g
0	0	198 ± 11[a]	94 ± 5	3.92 ± 0.26	2.70 ± 0.20	118 ± 7	1.06 ± 0.17	0.24 ± 0.02	56 ± 5	2.00 ± 0.14	0.82 ± 0.16	1.48 ± 0.59
0	5	202 ± 10	98 ± 5	5.30 ± 0.24	3.49 ± 0.18	153 ± 6	1.50 ± 0.16	0.39 ± 0.02	77 ± 4	2.05 ± 0.13	0.48 ± 0.16	4.29 ± 0.51
35	0	215 ± 10	107 ± 5	5.91 ± 0.26	3.75 ± 0.19	156 ± 7	1.77 ± 0.17	0.42 ± 0.02	76 ± 5	1.78 ± 0.13	0.86 ± 0.15	3.61 ± 0.53
35	5	218 ± 10	106 ± 5	6.29 ± 0.24	4.24 ± 0.18	176 ± 7	1.83 ± 0.17	0.48 ± 0.02	77 ± 4	2.52 ± 0.13	1.22 ± 0.14	2.76 ± 0.51
Analyses of Variance – P values												
Si		NS	0.04	0.0001	0.0001	0.0001	0.004	0.0001	0.03	NS	0.01	NS
Arg		NS	NS	0.001	0.002	0.0003	NS	0.0001	0.01	0.005	NS	NS
Si x Arg		NS	NS	0.05	NS	NS	NS	0.03	0.03	0.02	0.03	0.001

[a] Least square (LS) means ± standard error LS means.

vertebra when silicon was inadequate in the diet. Although the calcium and phosphorus concentrations were not affected by the addition of arginine to the diet, potassium and sodium concentrations in the vertebra were significantly elevated when additional arginine was fed. An interaction between silicon and arginine also affected the vertebral potassium concentration. Arginine supplementation elevated the potassium concentration in the vertebra more markedly in the silicon-deprived rats than in the silicon-adequate rats.

The concentrations of the trace minerals of vertebra with the exception of molybdenum and germanium reflect the nutritional status of silicon. Vertebra concentrations of zinc, copper, manganese, and iron were depressed by silicon deprivation. On the other hand, zinc, iron, manganese, and molybdenum concentrations were elevated when supplemental arginine was fed. Manganese, iron, and molybdenum concentrations were also affected by a significant interaction between silicon and arginine. When silicon was low in the diet, the addition of arginine enhanced the concentrations of manganese and iron in the vertebra to a greater extent than when dietary silicon was adequate and arginine enhanced the concentration of molybdenum to a greater extent in silicon-adequate than silicon-deficient vertebrae. An interaction between silicon and arginine affected the vertebral silicon concentration. Silicon deprivation decreased the silicon concentration in the vertebra only when arginine was supplemented to the diet. Another way of looking at the interaction is that arginine added to the silicon-adequate diet increased the vertebra silicon concentration but decreased the silicon concentration in rats fed the silicon-inadequate diet. Germanium in the vertebra was affected similarly to that found with the femur. When dietary silicon was adequate, the germanium concentration was lower in the arginine-supplemented rats than in the unsupplemented rats. When dietary silicon was inadequate, arginine supplementation increased the vertebral germanium concentration.

DISCUSSION

The plasma and vertebral silicon findings indicate that silicon deprivation of rats was achieved. Interestingly, arginine supplementation seemed to increase the difference in plasma and vertebral silicon concentrations between silicon-deprived and silicon-supplemented rats, but this effect was not always associated with an enhanced silicon effect on other variables examined. Another finding suggesting that the low-silicon diet induced a silicon deficiency was that it resulted in a lower final body weight. Growth depression has been proposed as evidence for the essentiality of silicon because this was one of the effects of silicon deprivation of chicks *(11).* Schwarz and Milne *(12)* also reported that growth was increased by feeding 50 mg Si/100 g diet, an amount 20 times higher than the silicon-supplemented diet used in the present experiment. It should be

noted, however, that the growth depression of the silicon-deprived rats was not very marked, and a significant growth depression has not always been found in other studies performed by us. Perhaps the severity of the silicon deprivation or the amount of silicon supplemented to make the diet adequate determines whether a significant growth effect is seen. The dietary arginine manipulations affected neither growth nor the liver weight/body weight ratio responses to the change in dietary silicon. Thus, physiological changes in somatic growth induced by silicon deprivation apparently are not altered by high dietary arginine.

Arginine supplementation did increase the kidney weight/body weight ratio slightly. This effect probably can be associated with the need to dispose of extra nitrogen and/or histological changes caused by the metabolism of the extra arginine. Evidence that the extra arginine resulted in altered nitrogen metabolism is that supplemental arginine increased plasma creatine; arginine is involved in the synthesis of creatine. L-Arginine also serves as the substrate for nitric oxide synthase, in addition to being the precursor of proline and polyamines. These metabolites of arginine can cause some fibrosis in the kidney; the severity of the fibrosis apparently is related to the arginine dose *(13).* Because blood urea nitrogen was not significantly affected in the present experiment, the increased kidney weight/body weight ratio apparently was not indicative of a notable kidney dysfunction.

One finding indicating that silicon is important in bone formation and remodeling is that plasma alkaline phosphatase is altered by dietary silicon intake. In the present experiment, plasma alkaline phosphatase was lowest in the rats fed adequate silicon; these rats apparently also had the best bone mineralization, as indicated by the mineral analyses in Tables 2 and 3. The finding of elevated plasma alkaline phosphatase in the silicon-deprived rats probably reflected that bone mineralization had not progressed as rapidly as in rats fed adequate silicon. Carlisle *(14)* stated that silicon is needed for the initiation of bone mineralization. We *(15)* found that mineralization of ectopic bone measured by ^{45}Ca incorporation was poorer under silicon-deficient than silicon-adequate conditions.

In addition to Carlisle *(3),* other research groups, including Watkins and Southern *(16)* and Leach et al. *(17),* using zeolite A have found that pharmacologic or physiologic intakes of silicon promote bone formation in chicks. Eisinger and Cairet *(18)* found that silicon was more potent in stimulating bone formation than some drugs, such as ethidronate, used in the treatment of osteoporosis. Rico et al. *(19)* reported that a supplement of 500 mg Si/kg diet fed to ovariectomized rats significantly increased the longitudinal development of the femur and prevented vertebral and femoral bone mass loss. Hott et al. *(20)* found that treatment of mature ovariectomized rats with 1 mg/kg/d of a soluble organic silicon compound (silanol) prevented trabecular bone loss and proposed that this finding was the result of both reduced bone resorption and increased bone formation. A soluble salt of silicon was also reported to increase trabecular bone

volume in humans *(21).* Although these studies show that supplemental silicon has beneficial effects on bone, further evidence is needed to state that silicon deprivation can lead to decreased bone mass or bone loss that can be prevented by nutritional or physiological intakes of silicon. The mineral composition findings in the present study provide some evidence for this. For example, the decrease in femoral calcium and vertebral phosphorus concentrations caused by feeding a diet low in silicon was prevented by supplementing the diet with 35 mg Si/kg.

The trace element findings also indicate that nutritional or physiological amounts of silicon are important in bone formation. Several trace elements have been shown to be involved in bone development or remodeling. For example, Ott and Asquith *(22)* found that several trace elements, including iron, manganese, and iodine, in addition to copper and zinc, were critical for bone mineralization in horses. Most of these minerals are involved in reactions producing an organic matrix upon which calcification occurs. The finding in the present study that silicon deprivation decreased copper, manganese, and zinc concentrations in femur and vertebra suggest that nutritional amounts of silicon are important in bone formation through a positive effect on the organic matrix. This finding is consistent with those of Carlisle *(23),* who reported that silicon is needed for the normal formation of collagen and glycosaminoglycans in bone and cartilage and is essential for the development of mesenchymal embryonic tissue and the early stages of calcification *(24).*

Rico et al. *(19)* proposed that silicon could affect bone remodeling both through a stimulation of bone formation and an inhibition of bone resorption. The finding that silicon in zeolite form increased the proliferation and differentiation of osteoblast-like cells supports this proposal; this effect corresponded to an increase in mRNA levels of transforming growth factor-β (a proliferation mitogen for osteoblasts). Evidence that silicon can affect bone resorption has been provided by Schutze et al. *(25);* they found that zeolite inhibited osteoclast-mediated bone resorption in vitro.

It is possible that silicon could affect osteoclast activity through an effect on nitric oxide production from arginine. In macrophages, silica induces nuclear factor-κB (NF-κB) activation and nitric oxide production; this induction is inhibited by the nonselective nitric oxide synthase inhibitor, *N*-ω-nitro-L-arginine methyl ester (L-NAME). The multiprotein complex NFκB is an essential transcription factor for the induction of nitric oxide synthase (iNOS) *(26).* Nitric oxide apparently has a crucial role in regulating osteoclast activity. Feeding an inhibitor of nitric oxide formation from arginine decreased bone mass in growing rats that was associated with an uninhibited increase in osteoclast activity *(27).* These findings suggest that a low dietary intake of arginine would have more of an effect on the response to changes in dietary silicon than a high intake of arginine (such as that used in the present study) and thus would result in more variables affected by an interaction between silicon and arginine.

In a previous study *(6),* we found that supplementing germanium to diets low in silicon increased femur DNA concentrations. The tibia DNA and femur and vertebra germanium findings in the present study are consistent with germanium affecting DNA in bone. When the DNA concentration in bone was decreased (e.g., by arginine supplementation of silicon-adequate rats), the germanium concentration in bone was decreased. This finding suggests that germanium may be of physiological importance.

In summary, findings were obtained showing that nutritional or physiological amounts of dietary silicon have beneficial effects on bone mineralization. The findings counteract a major criticism of silicon research in the past; that is, nonphysiological amounts of silicon were used to show that silicon affects the bone mineralization process. The findings also provide further support to the contention that silicon is an essential nutrient of practical importance.

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